Supporting Information

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SI Text

SI Methods. ***Neurite outgrowth assays.*** Glass coverslips were coated with poly-DL-ornithine (P-Orn) in pH 8.5 borate buffer (0.5 mg/mL) for 2 h at 37 °C and 5% CO_2, followed by CS-A,-C,-E polysaccharides (Seikagaku), CSPGs derived from chick brains (Millipore), digested CSPGs treated with ChABC (Seikagaku; 4 mU ChABC per μg CSPG), or synthesized polymers (1) [polysaccharides and polymers at 1 μg/mL based on uronic acid content (2) in phosphate buffered saline (PBS; 1 mM KH_2PO_4, 155 mM NaCl, 3 mM Na_2HPO_4, pH 7.4)] for 2 h at 37 °C and 5% CO_2. For mixed polymer assays, the polymers were mixed at the given concentrations immediately prior to coating. DRGs were dissected from day 7 chick embryos, incubated in 0.125% trypsin w/ EDTA (Invitrogen) for 15 min at 37 °C, triturated to dissociate to single cell suspensions, and grown on the coverslips coated with the above-mentioned substrata. Cells were grown in a growth medium composed of DMEM/F12, 10% horse serum, 50 ng/mL NGF (Sigma-Aldrich), and Insulin-Transferrin-Selenium-X Supplement (Invitrogen) for 12 h. For CGNs, cerebella were dissected from P5-9 Sprague Dawley rats, incubated in 0.125% trypsin w/ EDTA for 15 min at 37 °C, triturated to dissociate to single cell suspensions, and purified on discontinuous 35%–60% Percoll gradient. For the signaling pathway inhibitor studies, inhibitors against EGFR (AG1478, 15 nM; Calbiochem), ROCK (Y27632, 5 μM; Calbiochem), and JNK (JNK Inhibitor II, 10 μM; Calbiochem) were added in solution at the start of culturing, and neurons were grown for 24 h in DMEM/F12, 1% FBS, and N1 supplement at 37 °C and 5% CO_2. For the antibody blocking studies, anti-CS-E, anti-CS-A (Seikagaku), or IgG control antibodies (0.1 mg/mL) were added at the start of culturing to chick E7 DRGs, which were cultured as described above on glass slides with a substratum of P-Orn or CSPGs (0.5 μg/mL) for 12 h.

For inhibition studies using CSPGs derived from *Chst15*$^{-/-}$ mice (3), 96-well Poly-D-Lysine Cellware Plates (BD BioCoat™) were coated with CSPGs in PBS overnight at 37 °C and 10% CO_2. The plates were then washed with PBS and coated with laminin (Invitrogen; 10 μg/mL) in Neurobasal medium (Invitrogen) for 2 h at 37 °C and 10% CO_2. DRGs were dissected from P8 wild-type (WT) mouse pups, incubated in 0.125% trypsin w/ EDTA for 15 min at 37 °C, followed by collagenase (Worthington; 4 mg/mL) for 15 min at 37 °C, triturated to dissociate to single cell suspensions, filtered using a 40-μm cell strainer (Fisher) to remove nondissociated cells, and seeded at approximately 2,000 cells per well. Cells were cultured for 2 d in Neurobasal medium supplemented with B27 and GlutaMAX™ (Invitrogen).

For inhibition studies using neurons from *PTPσ*$^{-/-}$ mice (4), Poly-D-Lysine Cellware Plates were coated with laminin (10 μg/mL) in Neurobasal medium for 2 h at 37 °C and 10% CO_2. DRGs were dissected from adult knockout (KO) mice or WT controls, dissociated, and cultured as described above. CS-E was biotinylated as described (5) and conjugated to streptavidin agarose beads (200 μg of CS in 400 μL PBS incubated with 100 μL agarose resin for 1 h at RT), which were then coplated with the cells (5 μg of 50% slurry per well). Unconjugated beads at the same concentration were used as a control. Cells were grown in Neurobasal medium supplemented with B27 and GlutaMAX™ for 2 d. For all neurite outgrowth experiments, we performed statistical analysis using the one-way ANOVA test (n = 50–200 cells per experiment), and results from at least three independent experiments were reported.

Growth cone collapse assays. DRG explants were dissected from E7-9 chick embryos and grown in DMEM/F12 medium supplemented with 10% horse serum, Insulin-Transferrin-Selenium-G Supplement, and NGF (50 ng/mL) on 8-well Lab-Tek® II CC2™ Slides (Electron Microscopy Sciences) that were coated with P-Orn in pH 8.5 borate buffer, followed by laminin (10 μg/mL) in PBS for 2 h at 37 °C and 5% CO_2. CGN explants were dissected from P7-9 rats, chopped with a razor blade into approximately 1-mm^2 pieces, and cultured on P-Orn-coated glass coverslips in DMEM/F12 medium supplemented with 10% horse serum, 5% FBS, and N1 supplement. After 24 h, explants were treated with the indicated polysaccharides or glycopolymers (10 μg/mL based on uronic acid content in media; initial stock 200 μg/mL in PBS) for 30 min. *P*-values were determined using the one-way ANOVA test (n = 50–100 growth cones per experiment), and results from at least five independent experiments were reported.

Boundary assays. CS polysaccharides (1 mg/mL based on uronic acid content) were mixed with Texas Red (0.5 mg/mL; Invitrogen) in PBS, spotted at the center of P-Orn-coated coverslips (5 μL), and incubated for 2 h at 37 °C and 5% CO_2. Cerebella were dissected from P5-9 Sprague Dawley rats, incubated in 0.125% trypsin w/ EDTA for 15 min at 37 °C, triturated to dissociate to single cell suspensions, and purified on a discontinuous 35%–60% Percoll gradient. These cells were then cultured on the coated coverslips for 48 h. After immunostaining for neurite outgrowth, axons growing toward the boundary and within 10 μm distance of the boundary were evaluated. The percentage of axons that crossed the boundary over the total axons was quantified. *P*-values were determined using the one-way ANOVA test (n = 30–50 axons per experiment) and results from two independent experiments were reported.

Immunostaining and quantification. All neuronal cultures were fixed with paraformaldehyde, permabilized with 0.1% Triton X-100 in PBS, blocked with 1% BSA in PBS, and incubated with a mouse anti-βIII tubulin antibody (Sigma) overnight at 4 °C, followed by an Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody (Invitrogen) for 1–2 h at room temperature for neurite outgrowth and boundary assays, or by rhodamine phalloidin (Pierce) for 1 h at room temperature for growth cone collapse assays. Cells were imaged using a Nikon TE2000-S fluorescent microscope or Zeiss LSM Pascal, and neurite outgrowth was quantified using NIH software Image J or MetaMorph software. Statistical analysis was performed using the one-way ANOVA test (n = 50–500 cells per experiment), and results from at least three independent experiments were reported.

Protein expression and binding assays. For pull-down assays, full-length mouse *PTPσ* (Open Biosystems) was ligated into a pcDNA vector (Invitrogen) modified to fuse a myc-His tag to the 5′ end of the insert. COS-7 cells were transfected using Lipofectamine (Invitrogen) and lysed 2 d after transfection with 1% Triton X -100 in PBS containing a protease inhibitor cocktail (Roche). Lysates were then incubated with streptavidin agarose resin (Pierce; 30 μL) with end-over-end mixing for 1 h at 4 °C to reduce nonspecific binding. The supernatant was collected, added to 30 μL of either CS-C or CS-E streptavidin agarose resin, and incubated with end-over-end mixing for 4 h at 4 °C. The supernatant was removed, and the resin was washed three times with PBS (500 μL). Resin was boiled with 2X loading dye (30 μL of

100 mM Tris, 200 mM DTT, 4% SDS, 0.10% bromophenol blue, 20% glycerol), and the eluate was resolved by SDS-PAGE and transferred to PVDF membrane. PTPσ-myc was detected by immunoblotting with an anti-myc antibody (Cell Signaling) following the manufacturer's protocol. For ELISA and microarray assays, the extracellular domain of PTPσ was ligated into a pcDNA vector that had been modified to append a human Fc domain and myc-His tag to the expressed protein. HEK293T cells were transfected using Lipofectamine, and the conditioned media was collected and used for ELISA or subjected to Ni-NTA agarose purification for carbohydrate microarray assays.

ELISA and dot blot assays. To assay for PTPσ binding, PTPσ-Fc was incubated in 96-well protein A-coated plates (Pierce) overnight at 4 °C. The plates were washed with PBS containing 0.05% Tween-20 (PBST) and then incubated with biotinylated CS-A, CS-C, or CS-E polysaccharides in PBS for 2 h at room temperature. For the antibody blocking study, biotinylated CS-E (10 nM in PBS) was preincubated with the CS-C antibody (6) or CS-E antibody (10 μM) for 1 h at RT. The plates were then blocked with 1% BSA in PBS for 30 min at room temperature, incubated with horseradish peroxidase-conjugated streptavidin (Pierce; 1:25,000) for 1 h, and developed with TMB substrate (3,3′,5,5′-tetramethylbenzidine; Pierce) for 20 min and quenched with 2 M H_2SO_4. The absorbance at 450 nm was recorded on a PerkinElmer Victor plate reader. Experiments were performed in triplicate, and data represent the mean ± SEM, error bars.

For CS-E antibody binding to CSPGs, CSPGs (10 μg/mL; 25 μL) were incubated in a Nunc Maxisorp 384-well plate for 2 h. After blocking with 3% BSA in PBS, the anti-CS-E antibody (at the indicated concentrations in 1% BSA in PBS) was incubated in each well for 2 h. For CS binding assays, streptavidin (20 μg/mL; 50 μL) was absorbed in each well for 1 h, followed with biotinylated CS (20 μg/mL; 50 μL) for 1 h. After blocking with 3% BSA in PBS, the anti-CS-E antibody (25 μL of 20 μg/mL or indicated concentrations in 1% BSA in PBS) was incubated in each well for 2 h. Following incubation with horseradish peroxidase-conjugated anti-mouse IgG secondary antibody, the plates were developed and analyzed as described above. Dot blot assays for binding of CS-E Ab to CS polysaccharides were performed as described previously (7).

Microarray assays. Microarrays were generated as described previously (8). Arrays were blocked with 10% FBS in PBS with gentle rocking at 37 °C for 1 h, followed by a brief rinse with PBS. PTPσ-Fc, EphA2-Fc (R & D Systems), or Fc was reconstituted in 1% BSA in PBS, added to the slides in 100 μL quantities at a concentration of 1 μM, and incubated at room temperature for 3 h. The slides were briefly rinsed three times with PBS, and then incubated with a goat anti-human IgG antibody conjugated to Cy3 (Jackson ImmunoResearch; 1:5,000 in PBS) for 1 h in the dark with gentle rocking, and scanned at 532 nm using a GenePix 5000a scanner. Fluorescence quantification was performed using GenePix 6.0 software (Molecular Devices). Binding of the CS-E antibody was evaluated using 100 μL of a 1 μg/mL (or approximately 7 nM) solution of antibody and a goat anti-mouse IgG secondary antibody conjugated to Cy3. Experiments were performed in triplicate, and the data represent the average of 10 spots per concentration averaged from the three experiments (±SEM, error bars).

Mass spectrometry analysis. Brains were dissected from P7-P9 Sprague Dawley rats, homogenized in 0.32 M sucrose with protease inhibitors (Roche), and centrifuged at 1,000 × *g* for 10 min. The supernatant was collected, and then centrifuged at 10,000 × *g* for 20 min. The pellet was discarded, and the supernatant was centrifuged again at 12,000 × *g* for 30 min. This supernatant was then ultracentrifuged at 200,000 × *g* for 1 h, and the pellet was saved and homogenized again in 0.32 M sucrose with protease inhibitors. The supernatant was again ultracentrifuged at 200,000 × *g* for 1 h and the pellet was saved, solubilized in PBS containing 1% Triton X-100 with protease inhibitors, and centrifuged at 12,000 × *g* for 15 min. The final supernatant was obtained as the membrane protein-enriched fraction and incubated with CS-E or unsulfated CS conjugated to streptavidin agarose resin (described above) overnight at 4 °C. The resin was washed with PBS, and the PBS was collected and measured until the OD_{280} was less than 0.05. The bound proteins were then eluted with PBS containing 500 mM NaCl. The eluted proteins were then dialyzed into PBS and subjected to SDS-PAGE. The resulting gel was stained with Coomassie Brilliant Blue, and the band at 206 kDa was cut out, subjected to tryptic digestion, and analyzed by liquid chromatography-mass spectrometry (LC-MS) analysis as reported (9).

Surface plasmon resonance. All experiments were performed on a Biacore T100 at 25 °C using a Sensor Chip CM5 with a running buffer composed of 0.01 M Hepes, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05% Surfactant P20 (HBS-EP+). To analyze the binding of the CS-E antibody to the CS-E tetrasaccharide, both control and active flow cells were exposed to a 1:1 mixture of *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) for 3 min at a flow rate of 10 $\mu L \cdot min^{-1}$. Next, 5 mM carbohydrazine was injected at the same flow rate for 7 min. Ligand was covalently attached to the surface by injecting a 0.5 mM solution of synthetic CS-E tetrasaccharide bearing an aldehyde group on a reducing-end linker, prepared as previously described (7), onto the active flow cell briefly at a high flow rate (10 s, 60 $\mu L \cdot min^{-1}$), followed by a 20 min injection of 0.1 M sodium cyanoborohydride in 0.1 M sodium acetate pH 4.0 at 2 $\mu L \cdot min^{-1}$. Because of the low molecular weight of the CS-E tetrasaccharide, it was not possible to observe the amount of ligand bound to the surface. Instead, 500 nM of the CS-E antibody was injected into both the control and active flow cell to test the response. The amount of ligand was increased accordingly until an adequate response was observed. The kinetics of the CS-E antibody/CS-E tetrasaccharide interaction was determined by 300 s injections of the CS-E antibody at 30 $\mu L \cdot min^{-1}$. The dissociation was monitored for 900 s before the surface was regenerated with a 30 s injection of 6 M guanidine HCl. The resulting sensorgrams were fit to the bivalent analyte model. Affinity analysis was measured by injecting the antibody for 3,600 s at 5 $\mu L \cdot min^{-1}$. After 600 s, the surface was regenerated with a 60 s injection at 10 $\mu L \cdot min^{-1}$. The data were analyzed by plotting the response at equilibrium versus concentration and fitting the resulting curve to the Langmuir equation.

CSPG purification. Adult brains from *Chst15* knockout mice or WT controls were dissected and homogenized in PBS with 20 mM EDTA and protease inhibitors (Roche). The homogenates were centrifuged at 27,000 × *g* for 1 h at 4 °C. The supernatant was collected, and the pellet was homogenized and centrifuged as before, and the second supernatant was added to the first (total volume 8 mL). Urea (1 g) was added, and the supernatant was incubated at 4 °C for 1 h. For each brain, 2 mL of DEAE Sephacel beads was added to a column, and the supernatant was incubated with these beads for 2 h at 4 °C. The column was then drained, and washed with 50 mM Tris HCl, pH 7.5, 2 mM EDTA, 2 M urea, 0.25 M NaCl. The CSPGs were eluted with the same buffer, with 0.75 M NaCl. The eluate was concentrated using 50 kDa filter columns (Amicon), and dialyzed into PBS. Protein concentrations were determined using the carbazole assay with commercial CSPGs (Millipore) as a concentration standard.

RhoA activation assay. COS-7 cells were cultured in DMEM supplemented with 10% FBS. The cells were serum starved overnight, and then the medium was removed and replaced with serum-free medium containing CS-E polysaccharides or CSPGs (10 μg/mL). After 10 min, the cells were lysed, and RhoA activation was determined using the G-LISA kit (Cytoskeleton).

Immunostaining of retinal and optic nerve sections. At 14 days post injury, mice were given an overdose of pentobarbital and were transcardially perfused with 4% paraformaldehyde (PFA). Eyeballs still attached with the optic nerve were dissected and post-fixed in 4% PFA overnight. Following cryoprotection with 30% sucrose in PBS, tissues were embedded in Tissue-Tek® OCT™ and serial sectioned for 10 μm along the longitudinal direction of the nerve. For immunofluorescence labeling, the sections were washed with PBS, preincubated in a blocking buffer (1% BSA, 0.3%TX-100 in PBS) and followed by sequential incubations with primary antibodies, including mouse anti-CS-E (1:200), goat-anti-CTB (1:4,000), or rabbit anti-βIII-tubulin antibodies (Invitrogen). The sections were then reacted with biotinylated anti-goat IgG (1:200) and visualized with Alexa Fluor 546-conjugated streptavidin (1:400) or with a Cy3-conjugated goat anti-mouse or -rabbit IgG. The retinal cryosections were mounted with Vectashield and visualized under a Nikon fluorescence microscope. To quantify the number of CTB-positive regenerating axons, the number of regenerating axons was counted at 125 μm stepwise from the crush site of the optic nerve. The total number of regenerating axons was estimated as described (10). To quantify the number of surviving retinal ganglion cells, the total number of βIII-tubulin positive cells was counted in at least 3 retinal sections per retina.

Optic nerve regeneration assay. Immediately after crush injury in the optic nerve of adult mice, gelfoam soaked in a solution containing control IgG, CS-E antibody (1.7 mg/mL), chondroitinase ABC (ChABC; 50 U/mL), or ChABC (50 U/mL) plus CS-E antibody (1.7 mg/mL) was placed around the crush site of the nerve and replaced after three days. To analyze whether CS-E contributes primarily to the growth inhibition associated with CSPGs and acts extrinsic to neurons to block nerve regeneration, we also compared the effects of the CS-E antibody on optic nerve regeneration in vivo with that of CPT-cAMP, which is reported to stimulate the intrinsic growth status of retinal ganglion cell axons. Thus, in other groups of mice, mice that received an intravitreal injection of CPT-cAMP (100 mM, 2 μL) alone or CPT-cAMP plus CS-E antibody (1.7 mg/mL) treatment placed around the crush site of the nerve were studied. To label retinal ganglion cell axons, 2 μL of a solution containing an anterograde axon tracer, CTB, was injected intravitreally 3 d before mice were killed. The extent of axonal regrowth was assessed 2 weeks after injury.

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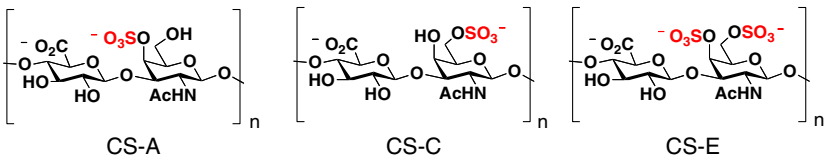


Fig. S1. CS polysaccharides are composed of 20–200 units of the repeating disaccharide D-glucuronic acid (GlcA) and *N*-acetyl-D-galactosamine (GalNAc). The sugar hydroxyls are variably sulfated to give rise to diverse sulfation patterns. Chemical structures of major sulfation motifs found in the mammalian nervous system: CS-A (GlcA-4SGalNAc), CS-C (GlcA-6SGalNAc), and CS-E (GlcA-4S, 6SGalNAc). $n = 20–200$.

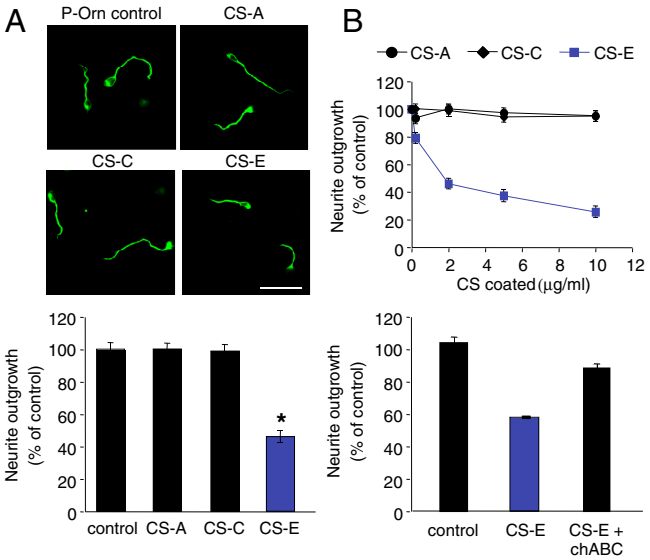


Fig. S2. Polysaccharides enriched in the CS-E sulfation motif (60% CS-E content), but not CS-A or CS-C (90% CS-A and CS-C content, respectively), inhibit the neurite outgrowth of cerebellar granule neurons (CGNs). (*A*) Dissociated P5-9 rat CGNs were cultured on a substratum of polysaccharides enriched in the CS-A, CS-C, or CS-E sulfation motifs (1 μg/mL) for 24 h. Cells were immunostained using an anti-βIII-tubulin antibody, imaged and quantified using the NIH software Image J. Representative images are shown on the top, and quantitation of the average neurite length (±SEM, error bars) from at least three experiments is shown on the bottom (One-way ANOVA, $*P < 0.0001$, relative to P-Orn control; $n = 50–200$ cells per experiment). (*B*) Polysaccharides enriched in the CS-E sulfation motif, but not the CS-A or CS-C motifs, inhibit CGN outgrowth in a dose-dependent manner (*Top*). Chondroitinase ABC (4 mU/μg) digestion abolishes the inhibitory properties of CS-E-enriched polysaccharides (1 μg/mL) in CGNs (*Bottom*).

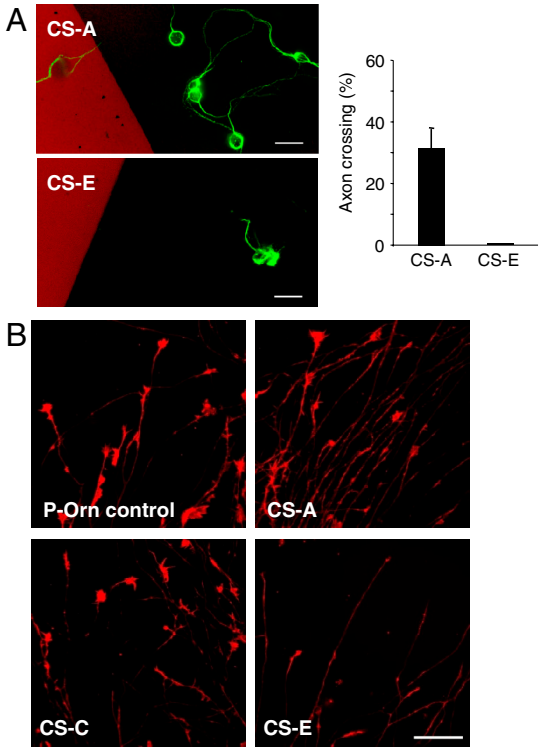


Fig. S3. Representative images of the (*A*) axon repellant activity of CS-A and CS-E polysaccharides at high sugar concentrations (10 mg/mL) and (*B*) growth cone collapse in rat P7-9 CGN explants induced by CS-E polysaccharides. Scale bars, 30 μm.

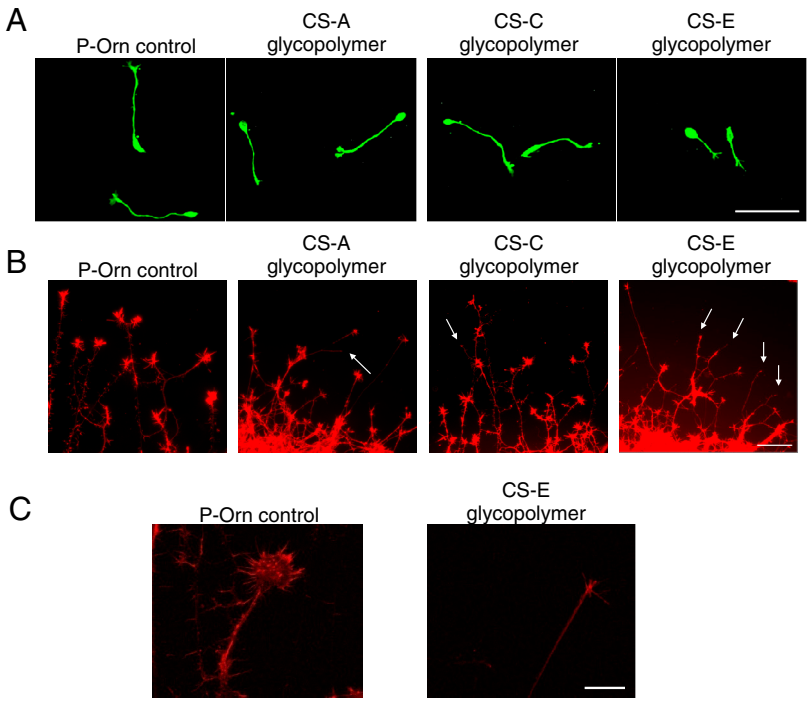


Fig. S4. Representative images of the (*A*) inhibition of chick E7 DRG outgrowth by the synthetic glycopolymers, and (*B*) growth cone collapse of chick E7-9 DRG explants induced by the synthetic glycopolymers. Arrows indicate collapsed growth cones. (*C*) Higher magnification images (60 ×) of intact and collapsed growth cones. Scale bars A and B: 100 μm, C: 20 μm.

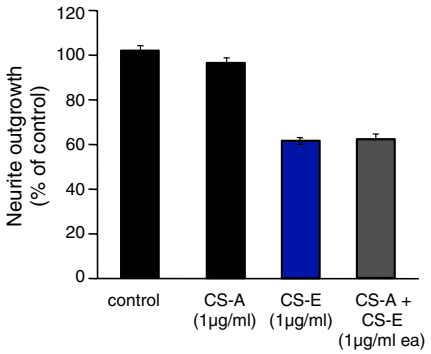


Fig. S5. A mixture of the CS-A and CS-E synthetic glycopolymers does not confer additional inhibitory properties compared to the pure CS-E glycopolymer. Dissociated E7 chick DRGs were cultured on the indicated substrates for 12–14 h. Cells were immunostained using an anti-βIII-tubulin antibody, imaged, and quantified using the NIH software Image J. Quantitation of average neurite length (±SEM, error bars) from three experiments (n = 100–150 cells per experiment) is shown.

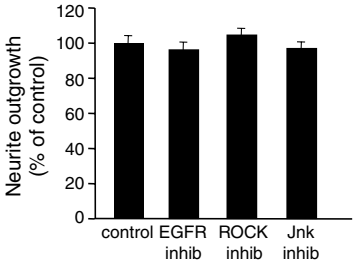


Fig. S6. Inhibitors to EGFR, ROCK, and JNK alone have no effect on CGN outgrowth in the absence of CS-E or CSPGs. Dissociated rat P5-9 CGN neurons were cultured on a P-Orn substratum in the presence or absence of inhibitors against EGFR (AG1478, 15 nM), ROCK (Y27632, 5 μM), and JNK (inhibitor II, 10 μM) for 24 h. Neurites were visualized by staining with an anti-β-tubulin III antibody, and quantitation of neurite outgrowth from at least three independent experiments is shown (n = 50–200 cells per experiment).

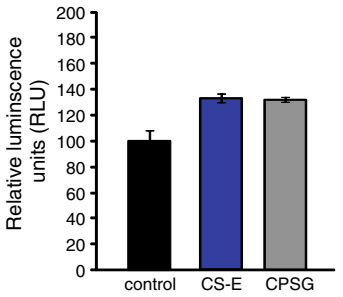


Fig. S7. CS-E and CSPGs activate RhoA. Serum starved COS-7 cells treated with CS-E polysaccharides or CSPGs (10 μg/mL) for 10 min. Cell lysates were standardized for total protein concentration and then added to a 96-well plate containing immobilized rhotekin-RBD, which binds the active (GTP-bound) form of RhoA. Bound RhoA was detected using a RhoA antibody followed by a horseradish peroxidase-labeled secondary antibody. Relative luminescence units (RLU) are plotted relative to that of the untreated control (cell medium alone) for two experiments.

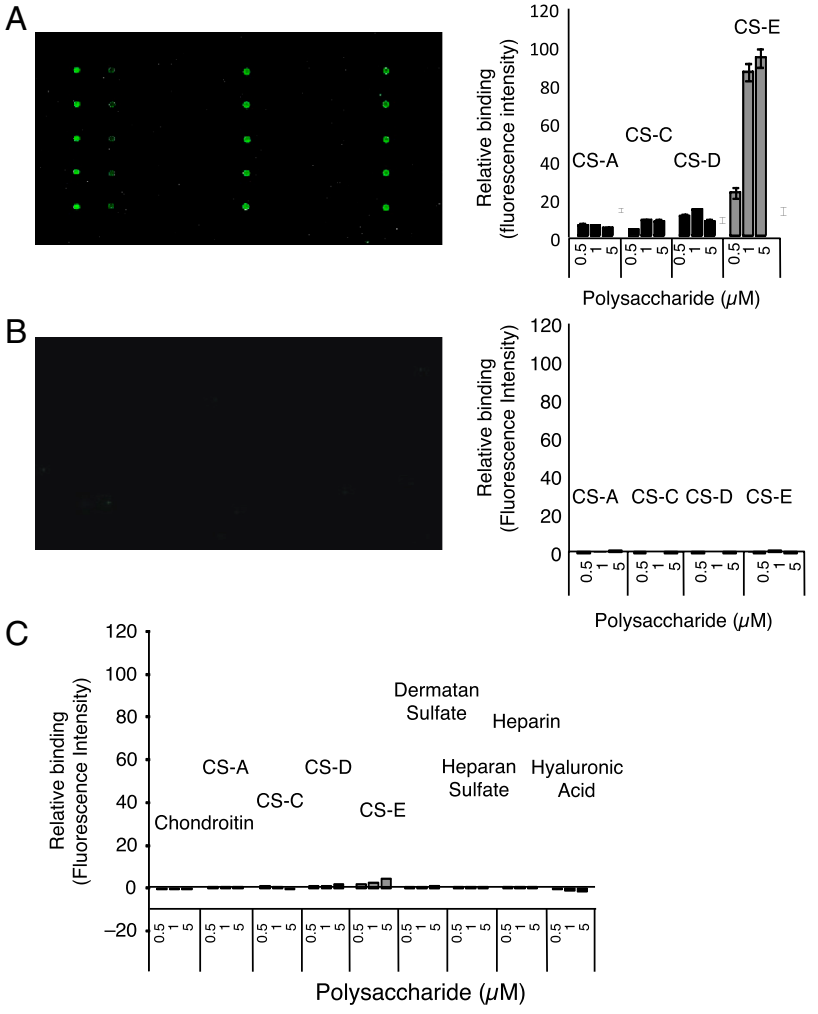


Fig. S8. PTPσ-Fc but not Fc alone or EphA2-Fc bind preferentially to CS-E-enriched polysaccharides. Representative portion of the microarray after binding to PTPσ-Fc (*A*, *Left*) or Fc control (*B*, *Left*). Quantitation from three experiments is shown on the right. Each bar (*A* and *B*, *Right*) represents an average of 10 spots per carbohydrate concentration. (*C*) EphA2-Fc binding to carbohydrate microarrays. Binding relative to PTPs-Fc is shown.

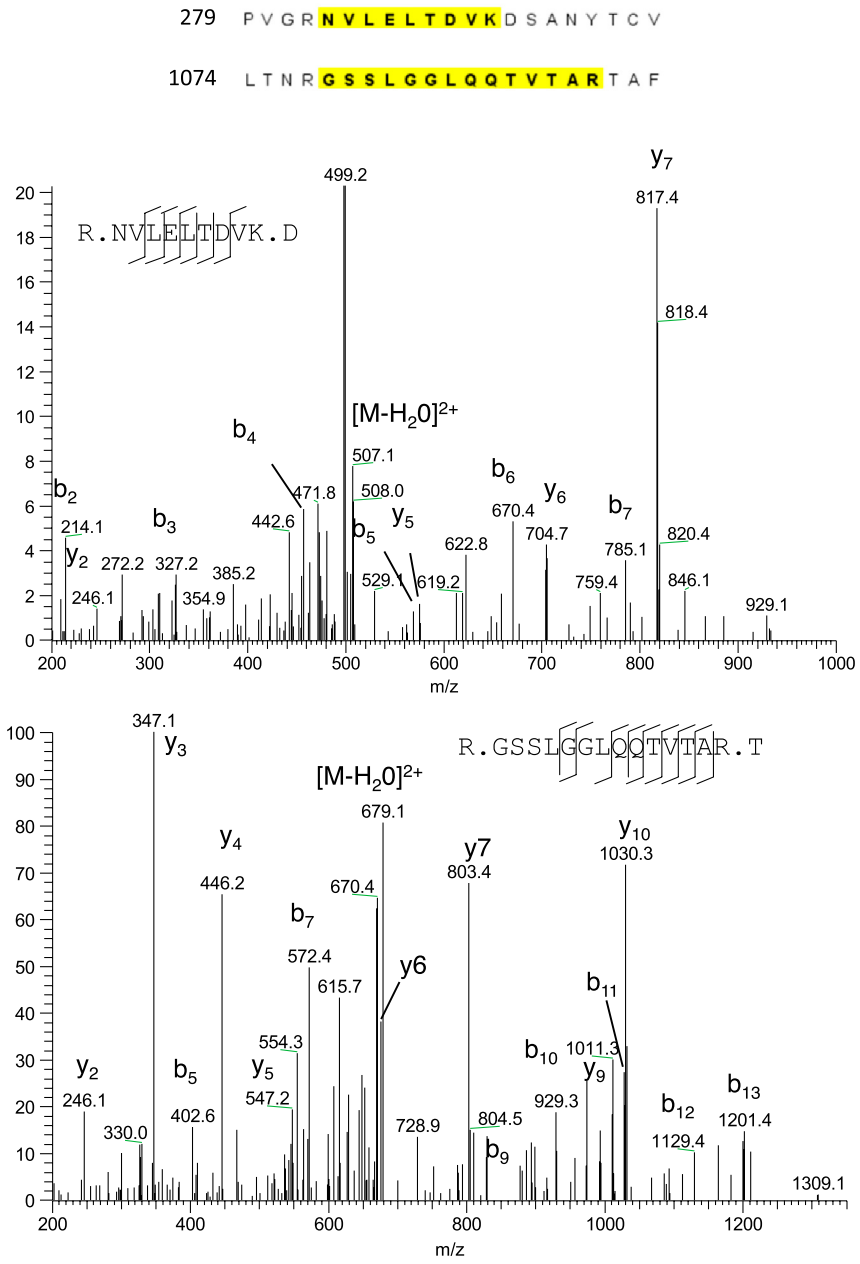


Fig. S9. PTPσ peptides identified by LC-MS/MS analysis. PTPσ from rat brain lysates was pulled down using CS-E and resolved by SDS-PAGE. In-gel tryptic digestion and LC-MS/MS analysis revealed two unique peptides within PTPσ. The annotated spectra from collisionally activated dissociation mass spectrometry (CAD-MS) of the peptides show the y and b fragment ions enabling identification.

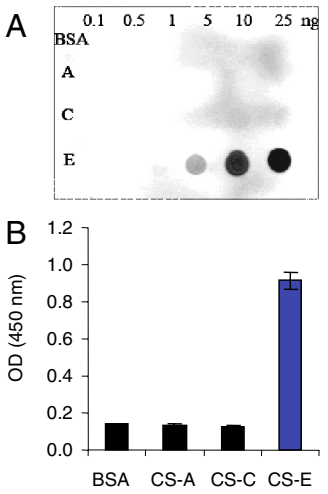
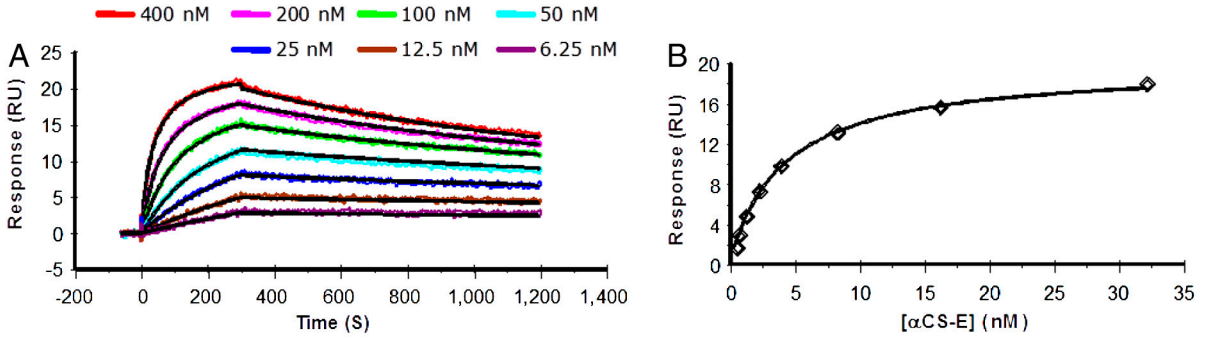


Fig. S10. The anti-CS-E antibody selectively binds to a pure CS-E tetrasaccharide and natural CS-E-enriched polysaccharides, whereas it does not bind to CS-A or CS-C tetrasaccharides or natural polysaccharides. (*A*) Tetrasaccharides containing pure CS-A, CS-C, or CS-E motifs were conjugated to bovine serum albumin (BSA) and spotted on nitrocellulose membranes at the indicated amounts. Binding of the antibody to the membrane was detected using an Alexa Fluor 680-conjugated goat antimouse secondary antibody. The anti-CS-E antibody bound in a concentration-dependent manner to the BSA-CS-E tetrasaccharide conjugate but did not bind significantly to BSA-CS-A, BSA-CS-C, or BSA alone. (*B*) Binding of the anti-CS-E antibody to biotinylated CS polysaccharides enriched in the CS-A, CS-C, or CS-E sulfation motifs. Biotinylated CS polysaccharides were absorbed on streptavidin-coated plates, and antibody binding to the plate was detected using a goat anti-mouse secondary antibody conjugated to horseradish peroxidase. Experiments were repeated in triplicate.



C

k_{a1} ($M^{-1}s^{-1}$)	k_{d1} (s^{-1})	k_{a2} ($RU^{-1}s^{-1}$)	k_{d2} (s^{-1})	R_{max} (RU)	χ^2(RU^2)
$3.77 (\pm 0.00) \times 10^4$	$6.41 (\pm 0.01) \times 10^{-4}$	$4.71 (\pm 0.84)$	$11.9 (\pm 2.1)$	$22.6 (\pm 0.02)$	0.059

Fig. S11. Kinetic analysis of the interaction between the anti-CS-E antibody and CS-E tetrasaccharide by surface plasmon resonance. (*A*) The synthetic CS-E tetrasaccharide was covalently immobilized onto the surface via reductive amination chemistry (see *Materials and Methods*). Kinetics were monitored at 25 °C by injecting the CS-E antibody over the surface for 300 s at 30 μL · min^{-1} and recording the disassociation for 900 s before the surface was regenerated with 6 M guanidine HCl. The resulting sensorgrams were fit to the bivalent analyte model. According to this model, a surface-bound analyte can bind another ligand molecule with the free binding site. The kinetic parameters of the fit, with standard errors in parentheses, are tabulated in (*C*). The affinity was also measured by injecting the antibody over the surface for 3,600 s to give sufficient time to reach equilibrium. The response at equilibrium was plotted versus concentration to give a K_D of 4.3 nM (*B*).

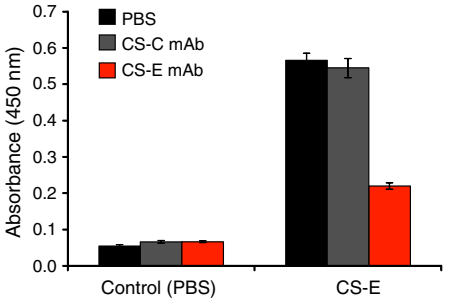


Fig. S12. The CS-E monoclonal antibody (mAb) attenuates binding of CS-E polysaccharides to PTPσ-Fc. PTPσ-Fc was immobilized in protein A-coated 96-well plates. Biotinylated CS-E (10 nM) in PBS was added in the presence of PBS (control), CS-C mAb (10 μM), or CS-E mAb (10 μM). Binding of CS-E was detected using a streptavidin-horseradish peroxidase conjugate. The experiment was performed in duplicate.

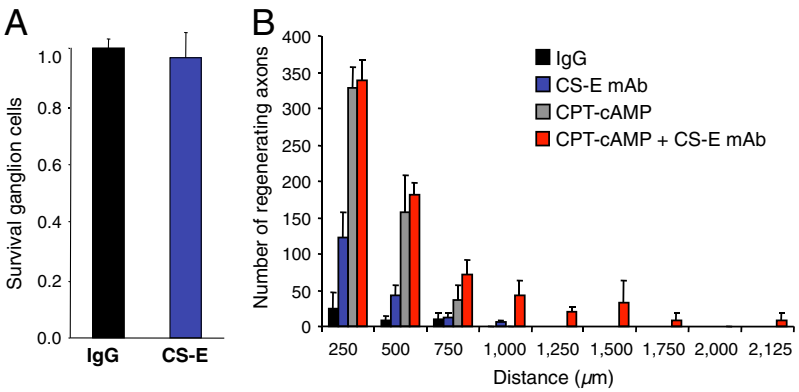


Fig. S13. The CS-E antibody does not affect the survival or intrinsic growth status of retinal ganglion cells. (*A*) Application of the CS-E antibody does not change retinal ganglion cell survival after optic nerve injury. Bar graph indicates relative survival of retinal ganglion cells in control IgG or CS-E antibody treated mice that were quantified at 14 days post optic nerve injury. (*B*) Comparison of axon regeneration in vivo induced by the CS-E antibody and/or CPT-cAMP. Retinal ganglion cell axons were counted at 125-μm intervals from the crush site from three nonconsecutive sections, and the number of fibers at a given distance was calculated as previously described (5) (±SEM, error bars). (ANOVA with Bonferroni posttests at each distance, $*P < 0.001$ as compared to controls; $n = 6$ for each group.